REPORT OF SOME EXPERIMENTAL WORK ON THE USE OF METHYLENE BLUE AND ALLIED DYES IN THE TREATMENT OF TUBERCULOSIS.*

STUDIES ON THE BIOCHEMISTRY AND CHEMOTHERAPY OF TUBERCULOSIS. VII.

LYDIA M. DEWITT.

(From the Otho S. A. Sprague Memorial Institute and the Pathological Laboratory of the University of Chicago.)

A preliminary report of experiments in the vital staining of tubercles was recently published by me,[1] showing which of the 20 or more dyes used penetrated the tubercle, which stained the tubercle bacillus, and which had any bactericidal power over that organism. In these earlier experiments, it was found that methylene blue penetrated the tubercle fairly well, that it stained the tubercle bacillus sharply and had *in vitro* some bactericidal power over these organisms and a considerable inhibitory action over their growth. These data being settled, it seemed worth while to try to determine how much, if any, therapeutic influence this dye had over the disease in question.

Methylene blue was one of the first dyes used *intra vitam*, as well as one of the earliest used in therapeutics. Its use as a vital or supravital stain of the nervous system, first introduced by Ehrlich,[2] and later developed by numerous neurological investigators in the effort to increase our knowledge of the structure of the nervous system and especially of the peripheral nervous system, is too well known to need further mention.

It was early used also as a vital stain for the lower plant and animal life. Hieronymus[3] in 1893 succeeded in staining with methylene blue the vacuoles of yeast cells while they were still alive, and Przesmycki[4] in 1894 with the same dye stained cell granulations in protozoa. Ruzicka[5] in 1904 stained bacteria and their intracellular granules with methylene blue and also fungi and leukocytes of different animals, observing in the stained cells extrusion of processes, division of granules, and other changes which he interprets as indicating the continued life of the cell. Methylene blue is, however, more or less toxic to these lower forms of plant and animal life and for this reason was suggested as early as 1880 as an internal antiseptic. Ruzicka[6] notes that in experiments in which he used both neutral red and methylene blue, part

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[1] *Jour. Infect. Dis.*, 1913, 12, p. 68.

[2] *Deutsch. med. Wchnschr.*, 1886, 12, p. 49.

[3] *Deutsch. bot. Gesellsch.*, 1893, 11, p. 176.

[4] *Biol. Centralbl.*, 1894, 14, p. 620.

[5] *Ztschr. f. allg. Physiol.*, 1904, 4, p. 141.

[6] *Op. cit.*

of the granules in the living cells took the red stain and part the blue, while the nucleus was unstained. As soon as the cell began to lose its vitality, the nucleus began to stain blue and finally, in the dead cell, nucleus and all granules were blue. In 1891, Einhorn[1] showed that the urine of patients who had been treated with methylene blue was sterile and remained sterile for three weeks. In 1900, Chaleix-Vivie,[2] because of the common use of the dye in gynecologic work, tested its bactericidal action on *Staphylococcus albus*, streptococcus, *B. coli*, *B. subtilis*, and other bacteria most frequently found in the utero-vaginal tract, and found that only *B. subtilis* retained its vitality after 24 hours' exposure to a dilute solution of the dye. In 1913, Churchman[3] partially verified this work, at least so far as the inhibitory action of methylene blue was concerned, since he found, by his divided plate method, a certain selective action of methylene blue, tho not always uniform.

It was perhaps a natural result of the observation of the specific staining action of methylene blue on the nervous system and especially on the nerve fibers that this dye was introduced into therapeutics by Ehrlich and Lippmann[4] in 1891 as an analgesic. The *schmerzstillende Wirkung* ascribed to the dye by these observers led to its use in neuralgias, nervous headaches, muscular rheumatism, epilepsy, migraine, psychoses, pleuritic pains of tuberculosis, etc.

It was found by Müller[5] and also by Elsner[6] that the dye was largely excreted by the kidneys, Elsner finding from 40 to 50 per cent of the total intake excreted by the kidneys in the first three days, 4 to 8 per cent by the intestines, while the rest could not be recovered or accounted for. Müller's figures were a little higher, as he recovered about 70 per cent of the total intake. Since the renal excretion begins very early, the urine becoming bluish green in about 15 minutes after the ingestion of the dye, it was thought that both its bactericidal and its analgesic power might be invoked in the treatment of diseases of the kidney, bladder, and urethra. Accordingly ordinary pyogenic infections and also gonorrheal infections of the genito-urinary apparatus have been treated more or less effectually with methylene blue. It has also been shown to have some diuretic action and its rapid excretion makes it valuable in testing renal permeability and function. Dysentery and other infections of the digestive tract have also been treated with methylene blue.

In line with the bactericidal action of the dye, Ehrlich and Gutmann[7] in 1891 announced that methylene blue, which stained malarial plasmodia very well, had been used by them in the treatment of malaria; they showed that it relieved the symptoms of malaria and eventually caused the disappearance of the plasmodia from the blood. Other workers have used the dye in this disease with greater or less benefit and, while it does not replace quinin, it may be used with it or after it has failed to conquer the disease.

In 1891, about the time when Ehrlich and Lippmann reported the analgesic action of methylene blue, Einhorn[8] began to use the dye in the treatment of some eight cases

[1] *Deutsch. med. Wchnschr.*, 1891, 17, p. 620.

[2] *Compt. rend. Soc. de biol.*, 1900, 5, p. 242.

[3] *Jour. Exper. Med.*, 1913, 18, p. 187.

[4] Reviewed in *Berl. klin. Wchnschr*, 1907, 44, p. 233.

[5] *Deutsch. Arch. f. klin. Med.*, 1899, 63, p. 130.

[6] *Ibid.*, 1901, 69, p. 47.

[7] Reviewed in *Berl. klin. Wchnschr.*, 1907, 44, p. 233.

[8] *Op. cit.*

of pulmonary tuberculosis, partly with the idea that it might prove a good anodyne, checking the cough and relieving the pain, and partly because he thought that a portion of the dye might be excreted in the lungs and there exert its bactericidal power, killing the tubercle bacilli within the lung tubercle. He found that the sputum of these patients always contained the dye in the form of crystals or small granules, but it was never sterile. This is the earliest report found by me of the treatment of tuberculosis with methylene blue, but it has been used by others both in the treatment of pulmonary tuberculosis and as a local application in tuberculous pharyngitis, in tuberculous glands, and in irrigating tuberculous sinuses and cavities. During the last year, the use of methylene blue in the treatment of tuberculosis has been revived by a group of German laboratory workers and clinicians. The leader, Professor Gräfin v. Linden, states[1] that living tubercle bacilli are readily stained by methylene blue (either chlorid or iodid), but are not killed by the dye unless it has been reduced by iron hydroxid. They fail to grow after 24 hours' exposure to the iron-reduced dye. She also states that tuberculous guinea-pigs treated with the dye show in the lungs grayish nodules which become blue after exposure to the air, and that these stained nodules contain blue-stained tubercle bacilli. She also finds blue-stained bacilli in the caseous substance of tuberculous lymph glands. In her first report, six guinea-pigs were treated with methylene blue. The treatments began eight days after inoculation. The dose used was 0.2 to 0.3 c.c. of a 0.1 per cent solution, the dye being injected subcutaneously and the dose repeated every day at first and later not so often. Medicinally pure methylene blue hydrochlorid was used at first and later the hydroiodid was used instead. She says that the treatment caused healing of the initial sinus and abscess, increase of weight, lowering of fever, and prolongation of life, the controls dying in 15 to 18 weeks and the treated animals in 28 to 42 weeks. She claims that 50 per cent of the animals died from some other cause than tuberculosis and that one guinea-pig was perfectly cured and sterile, as shown by animal inoculation. All cases showed a tendency to healing as indicated by a more fibrous rather than caseous form of the disease. In her second series, the iodid of methylene blue was used instead of the chlorid, with results not essentially different from those attained in the first experiment. No other case of "perfect healing and sterilization" was reported. These animals were inoculated with 0.5 mg. of a two-months-old culture of human tubercle bacilli of an estimated virulence of 15 weeks. These small doses of cultures of low virulence were used expressly to hinder too rapid development of the disease and to allow more time for the dye to take effect. Slight as were her results, they were sufficient to encourage the use of the dye in the therapeutics of tuberculosis. At Finkler's and v. Linden's suggestion, Strauss[2] and Meissen[3] used the dye both with and without copper in a number of patients suffering with pulmonary tuberculosis and also in a number with external tuberculosis. Meissen's report on his cases of pulmonary tuberculosis were not especially encouraging, but Strauss, who usually employed the methylene blue combined with some copper compound, claims that it caused diminution of pain and healing of fistulas and abscesses. Selter[4] makes a controversial report, claiming that he worked with Finkler and also with v. Linden and received no credit for his part in the work. He also claims that v. Linden's results, as he saw them, and also the results obtained in his own experiments were not decisive and did not deserve so positive a report and recommendation as had been given by v. Linden.

[1] *Beitr. z. Klin. d. Tuberk.*, 1912, 23, p. 201; also *München. med. Wchnschr.*, 1912, 59, p. 2560.

[2] *Beitr. z. Klin. d. Tuberk.*, 1912, 23, p. 223.

[3] *Ibid.*, p. 215.

[4] *Ibid.*, 1912, 24, p. 261.

My own experiments with methylene blue had begun before the appearance of the German reports and have continued up to the present time. The first experiments were intended only to test the power of the dye to penetrate the tubercle and were reported, at least in part, in my preliminary communication. The first essential in the use of a drug or dye as a chemotherapeutic agent in tuberculosis is that it shall be able to penetrate the tubercle; better, that it shall be specific for the tubercle and be retained in it, and pile up there so as to attain a cumulative influence. Six guinea-pigs suffering with advanced tuberculosis were given subcutaneous injections of the dye, four receiving medicinally pure methylene blue (Merck) and two, Ehrlich's rectified methylene blue. The dye was used in 0.25 per cent and 1 per cent water solutions and 1–2 c.c. of the solution injected subcutaneously, one to six injections being given at intervals of two or three days. In all cases there was much infiltration and sloughing of the subcutaneous tissues and necrosis of the skin. In most cases at autopsy the tissues appeared unstained at first, but after exposure to the air and, better, after exposure to 10 per cent ammonium molybdate, the tubercles of the lungs and liver and spleen, and especially of the lungs, became blue. The normal tissue usually remained unstained, tho sometimes there was a general diffusion of the stain. In no case was I able to find tubercle bacilli stained blue, altho at times blue-stained granules were seen which could not be identified as intrabacillary granules. The caseous centers of tuberculous lymph glands generally, not always, remained unstained or less deeply stained than the periphery of the gland, and stained bacilli were never found in them. In this respect, I was less successful than v. Linden, who finds blue-stained bacilli both in the lung tubercles and in the caseous centers of glands. As a rule in my animals, the blue was present as a diffuse stain of the tubercles; still, many times, stained granules and sometimes stained nuclei were seen. Tho no quantitative estimate was made, there seemed to be no heaping-up of the dye in the tubercles, since they appeared as deeply stained in the animals that died after a single injection of 1 c.c. of a 1 per cent solution as in those that received six injections aggregating 5 c.c. of a 1 per cent solution. This is probably due to the fact that the dye is quite

rapidly excreted, as shown by Elsner and Müller, Elsner recovering about 60 per cent from the urine and feces within three days after a single injection and Müller recovering about 70 per cent. Elsner suggests that the amount not recovered either may be deposited somewhere or may be excreted in a leuko-form which cannot be reoxidized. The latter explanation seems to me the more plausible; at least, if it is deposited, it seems to be deposited in a form that cannot be reoxidized after three or four days, since animals dying more than three or four days after the last injection rarely showed any blue in the tissues even after exposure to the air and to 10 per cent ammonium molybdate. Two guinea-pigs with advanced tuberculosis were then fed cake pills containing each about 4 mg. of medicinally pure methylene blue. One of the pigs died after only two feedings, while the other received 18 feedings of 25 pills, or 100 mg. of methylene blue. At autopsy both showed tubercles which became blue after oxidation, very much as did the animals that had been injected, and without any of the unpleasant ulcerations which had been so dangerous a feature in the injected animals. The pills were made by mixing cup cake, water, a little olive oil, and methylene blue, dividing the mass into parts as nearly equal as possible, rolling each portion between the hands into a pill, and then drying. The method resembles that recommended by Ehrlich[1] for the administration of parafuchsin and other dyes. The pills were readily eaten by the guinea-pigs and it was noted as curious that they became especially fond of the pills and eager for them as the tuberculous process advanced, so that one could predict with a good deal of certainty the approaching death of the animal from the eagerness with which it ate the pills and begged for more.

BACTERICIDAL POWER OF METHYLENE BLUE.

Having determined that a dye or drug will penetrate a tubercle, the next question of importance is: Will it enter and destroy the tubercle bacillus? With a dye, the question of penetration of the organism is easily determined by the sharpness with which the individual bacilli are stained by a given strength and in a given time. The difficulty of staining the tubercle bacillus is too well

[1] *Op. cit.*

known to need comment. Miss Sherman[1] in this laboratory has shown conclusively that the ordinary fat-soluble dyes are very poor stains for the bacilli, tho they often stain masses of culture readily and sharply. In my preliminary report, I showed that only a few of the dyes studied by me were really good stains of the individual organisms, tho most of them stained masses of organisms very well. The different types of methylene blue which I had at that time used all had the power, even in 1 per cent water solutions, of staining the individual bacilli quickly and sharply. This fact is also noted by v. Linden and by Sherman. In my earlier communication it was shown also that even very dilute solutions of the dye added to culture media had power to inhibit the growth of the tubercle bacilli, and v. Linden says that 24 hours' exposure to the reduced dye kills the organisms so that they will not grow when planted on suitable media.

In this set of experiments, the method used by me for testing the bactericidal action of the dyes was as follows: A very dilute filtered suspension of virulent human tubercle bacilli in normal salt solution is made, such that there is the merest trace of opalescence in the filtrate. One drop of this suspension is mixed with 5 c.c. of sterile 1 per cent solution of the dye to be used and left, usually at incubator temperature, for 24 hours. One drop of the mixed dye and suspension is then diluted with 5 c.c. of normal salt solution and injected subcutaneously into a guinea-pig. Microscopic examination has shown that in this method of procedure we have very few clumps of bacilli, so that each individual bacillus is surrounded by the dye and may be easily penetrated by it and killed if it has any bactericidal power. In all cases examined in which any of the methylene blues were used, the organisms were found to be well stained.

Fourteen guinea-pigs were inoculated with tubercle bacilli which had been exposed for 24 hours to 1 per cent solutions of the different preparations of methylene blue, and Table 1 gives very briefly the results. As most of these animals were used for therapeutic experiments with different dyes after the local tubercle had developed, and as the treatment may in some cases have modified the results, the treatment used is indicated in column 4 of the table.

[1] *Jour. Infect. Dis.*, 1913, 12, p. 249.

Of this series, No. 2 was especially interesting. The animal remained well, its nutrition was unusually good, and it bore several litters of healthy young. Finally, 9 months after inoculation, it was

TABLE 1.

Dye Used	Time before Local Tubercle Developed	Time before Death	Treatment Used, If Any	Postmortem Findings
1. Ehrlich's rectified methylene blue....	None developed	9 months (killed)	None	No tubercles found. Animal normal
2. Medicinally pure methylene blue (Merck's).....	None developed	9 months (killed)	None	No local or general tuberculosis. Three encapsulated and apparently sterile nodules and some adhesions in abdomen
3. Medicinally pure methylene blue (Merck's).....	25 days	6 months	Methylene blue	Caseous gland in groin. Enlarged and necrotic spleen. No other involvement
4. Ehrlich's rectified methylene blue....	2.5 months	4 months	Silver trypan blue	Slight local and marked general tuberculosis
5. Ehrlich's rectified methylene blue....	2.5 months	3.5 months	Silver trypan blue	Small local tubercle. Liver and spleen necrotic. No other involvement
6. Ehrlich's rectified methylene blue....	46 days	75 days	Methylene blue (feeding)	Local and general tuberculosis
7. Medicinally pure methylene blue....	45 days, but disappeared	99 days	Selenium blue	No local tubercle. Spleen and liver large and necrotic. Few small tubercles in lung
8. Medicinally pure methylene blue....	45 days and developed slowly	93 days (killed)	Methylene blue (feeding)	Small local tubercle. Small tubercles in omentum. Very little other involvement
9. Medicinally pure methylene blue....	33 days and developed slowly	98 days	Lithium carmine	Very small local tubercle. Lungs filled with tubercles. None in liver and spleen
10. Methylene blue iodid	18 days	4 months	Congo red	Slight local and marked general tuberculosis
11. Methylene blue iodid	18 days	5 months	Congo red	Slight local and marked general tuberculosis
12. Methylene blue iodid	18 days	2.5 months	Congo red	Local and general tuberculosis
13. New methylene blue N.....	25 days	6 months	Methylene blue	Local and general tuberculosis
14. New methylene blue GG.....	32 days	133 days (killed)	Methylene blue	No local tubercle. Spleen soft, but no tubercles. Few necrotic areas in liver and lung

killed. Autopsy showed rolls of adipose tissue everywhere. No sign of malnutrition. None of the superficial glands were enlarged and all organs appeared normal, altho there were a few old adhesions around the spleen. Three sharply circumscribed, thick-walled

nodules filled with soft, cheesy, puruloid material were, however, found in the abdomen, one just under the anterior abdominal wall and the other two in the great omentum near the spleen. Smears from the interior substance of these nodules showed no acidfast bacilli and indeed no bacteria of any kind. Unfortunately no animal inoculations were made, but the findings seem to indicate that in this case some of the tubercle bacilli survived the treatment with methylene blue and remained capable of growth, but with virulence so weakened that no general infection resulted; the tuberculous process showed a marked tendency to healing and encapsulation and the organisms finally died out.

From Table 1, it may be seen that methylene blue in none of the preparations used can be depended on as a bactericidal agent. In one case all the organisms seem to have been killed, and in another, much attenuated. In all the tests, except those with methylene blue iodid, the local tubercle was slow in developing and generally remained small, while in some cases, either no local tubercle ever developed or one developed and later disappeared. It is difficult to draw conclusions from the very irregular time of death, since the animals, after the discovery of a local tubercle, were used for testing the therapeutic action of different dyes, some of which may have hastened and some delayed the exitus of the animal. It is to be noted that a slight or no local involvement is the rule and in many of the cases the general extension was so slight that one could scarcely ascribe the cause of death to the tuberculous involvement and must believe that some other cause intervened. Only one of the 14 animals, however, was entirely free from infection.

THERAPEUTIC EXPERIMENTS WITH METHYLENE BLUE.

In beginning my therapeutic experiments with methylene blue, it seemed to me necessary, because of the ulceration and sloughing caused by even the purest medicinal preparations of methylene blue which were employed in my earlier experiments, to use the dye in very dilute form and in very small doses. The first series of guinea-pigs subjected to treatment with the dye had been inoculated in experiments undertaken to test the bactericidal action of various dyes. The treatment was therefore begun relatively late in the

disease, since it was desired to find a palpable local tubercle before interrupting the disinfectant experiment. In all cases where no preparation is mentioned, Merck's medicinally pure methylene blue is the preparation used.

1. Treatment begun 35 days after inoculation. Subcutaneous injections of 0.5 to 1.0 c.c. of a 0.1 per cent solution were given at intervals of 2–3 days. Forty-eight injections were given, totaling 32 mg. of the dye. Death, 144 days after inoculation. Local glands enlarged and caseous. Spleen large and necrotic. Lungs and liver not involved. Tubercles deep blue.

2. Treatment begun 35 days after inoculation. Fifty-one injections given, totaling 54 mg. of methylene blue. Died 152 days after inoculation. A few enlarged and caseous glands in groin. Spleen, liver, and lungs contained a few miliary tubercles. General tuberculosis not advanced. Acute peritonitis, pericarditis, and pleurisy existed and were probably the cause of death.

3. Treatment begun 35 days after inoculation. Killed on one hundred and thirty-third day, after 45 injections containing 39 mg. of the dye had been given. A foul-smelling, necrotic ulcer in abdominal wall from action of dye. No local tubercles. Spleen large and soft, but showing no tubercles or necrotic areas. A very few small necrotic areas in liver and lungs. Tuberculous process very slight. (These three animals had been inoculated with tubercle bacilli which had been exposed for 24 hours to methylene blue. Hence the low virulence of the infection may be due to this fact rather than to the after treatment with the dye.)

4. This pig was inoculated with a culture which had been exposed for 24 hours to 1 per cent neutral red. No definite tubercles were found until the forty-seventh day after inoculation, but a curvature of the spine in the cervical region and partial paralysis developed on the fortieth day. Treatment with methylene blue was begun on the forty-seventh day, at which time the pig was so weak and ill and emaciated that it was thought to be near death. However, it improved rapidly after the methylene blue treatment was begun; it gained in weight and general condition; the curvature and paralysis both improved, altho the head always tended to turn to one side. About 50 injections were given, making approximately 53 mg. of the dye. The animal died 186 days after inoculation, its nutrition still being excellent. The sloughing of the skin and subcutaneous tissues had become so bad that no injections were given during the last two weeks of life. One gland in groin was enlarged, but hard, not caseous. The liver was pale and hard and showed a few greenish areas of necrosis. The spleen was large and soft and imbedded in a mass of partly coagulated blood. The lungs appeared normal. Death seemed to have been caused by rupture of the spleen and hemorrhage.

5. Trypan red was the disinfectant used in this case and a local tubercle was found 19 days after inoculation. Methylene blue treatment was begun 35 days after inoculation. Forty-three injections, making 42 mg. of methylene blue, were given. The pig died 131 days after inoculation, showing a large group of caseous glands in the groin, spleen and liver large and necrotic, and lungs filled with larger and smaller tubercles, many of which contained cavities.

6. This pig was inoculated with human tubercle bacilli which had been exposed for 24 hours to 1 per cent bluish eosin. Treatment with methylene blue was begun 35

days later. Forty-three injections were given, totaling 42 mg. of methylene blue. Death occurred 130 days after inoculation. Several glands in the groin were enlarged and caseous. The liver and spleen were large and necrotic. The lungs were filled with tubercles, many containing cavities.

7. This pig developed paralysis of the leg of the infected side after 40 days, but no local tubercle until 47 days after inoculation. Methylene blue was begun on the forty-eighth day and continued until death, which occurred 105 days after inoculation. Glands in groin were large and hard, but not caseous. Liver and spleen were large and necrotic. Lungs were filled with tubercles, some large, some small, the larger ones generally containing cavities.

The next series of animals were inoculated with a large dose of virulent culture of human tubercle bacilli. Treatment was begun two weeks after inoculation, the same method of treatment and the same doses being used as in Series 1. The four untreated controls had all developed local tubercles by the twenty-first day and died of local and generalized tuberculosis in 58, 126, 138, and 231 days.

1. Forty-two injections were given, making a total of 30 mg. of methylene blue. Death occurred 112 days after inoculation. There had been much necrosis and sloughing of skin and subcutaneous tissue. One gland in groin was enlarged and caseous. Liver hard and filled with tubercles and necrotic areas. Spleen moderately enlarged and almost entirely necrotic. Lungs dotted with young tubercles. Abdomen filled with bloody fluid.

2. Forty injections, making a total of 27 mg. of the dye, were given. Died on the one hundred and fifth day after inoculation. Caseous gland in groin; liver and spleen large and necrotic; lungs filled with tubercles. Pleural cavity filled with a serous exudate.

3. Forty-two injections, making a total of 33 mg. of methylene blue. Death on one hundred and twelfth day. Large group of caseous glands in groin. Liver very sclerotic with necrotic border. Spleen large and necrotic. Lungs filled with tubercles. Abdomen filled with clear fluid.

4. Thirty-six injections, making in all 24 mg. of methylene blue. Death 89 days after infection. Large caseous gland in groin. Liver not involved. Spleen slightly enlarged and shows a few miliary tubercles. Lungs show no tubercles. Pleural cavity filled with a thick, white, turbid, semi-solid exudate, covering lung surface and pericardium. No acidfast organisms were found in this exudate.

5. Forty-eight injections, in all 34 mg. of methylene blue. Death 134 days after infection. Two slightly enlarged, hard, not caseous glands in groin. Spleen large and filled with tubercles. Liver hard and pale. Lungs showed many tubercles. Pleural cavities filled with serous exudate.

As I had earlier found that the so-called new methylene blues penetrated the tubercle very well and also stained the tubercle bacilli and inhibited their growth, seven of the new methylene blues were used to treat as many guinea-pigs, the treatment being begun, as in the last experiment, two weeks after infection.

1. New methylene blue R. Forty-three injections were given, making a total of 47 mg. of the dye. Death occurred on the one hundred and twenty-ninth day. Slightly enlarged gland in groin and miliary tubercles in lungs, liver, and spleen.

2. New methylene blue GB. Thirty-seven injections, equaling 25 mg. of dye. Death on one hundred and sixth day. Caseous glands in groin. Liver and spleen enlarged and necrotic. Lungs free from tubercles.

3. New methylene blue N. Forty-five injections, making about 49 mg. of the dye. Death 133 days after inoculation. No local tubercle. Liver pale and hard; no tubercles. Spleen large, white, and necrotic. Lungs firm and dotted with tubercles.

4. New methylene blue NSS. Thirty-six injections, or 36 mg. of the dye. Died 100 days after infection. Caseous glands in groin. Liver and spleen and all abdominal organs appear normal. Lungs show no tubercles. Pleural cavity filled with a thick, yellowish-white, semi-solid exudate. No acidfast organisms in smears from this exudate, but many cocci and short, thick bacilli.

5. New methylene blue GG. Forty-five injections, making in all 44 mg. of the dye. Injections stopped some weeks before death, because of the bad condition of the animal. Died 140 days after infection. Slightly enlarged, but not caseous glands in groin. Abdominal and thoracic cavities filled with thick, turbid exudate. Lungs and liver contained a few small tubercles, but death was evidently due to acute pyogenic infection causing peritonitis and pleurisy.

6. New methylene blue NX. Much infiltration and necrosis of skin. Thirty-one injections, containing 34 mg. of the dye. Died 86 days after infection. Local gland slightly enlarged and hard, but not caseous. Liver and spleen large and very necrotic. Large tubercles in lungs, many containing cavities.

7. New methylene blue 3R. Thirty-five injections, making 39 mg. of the dye. Died 99 days after infection. Glands in groin large and caseous. Spleen and liver large and necrotic. Lungs showed a few tubercles.

Since v. Linden reports that she finds the iodid of methylene blue to have certain advantages over the chlorid, among them being that it is less irritant, I used some pure methylene blue iodid on a series of animals. This dye was 30.94 per cent iodin, and since iodin was found by Wells and Hedenburg[1] to penetrate the tubercle especially well, it was thought possible that this factor might cause the dye to penetrate the tubercle better and at the same time increase its bactericidal and therapeutic power. So far as its bactericidal power is concerned, however, it has proved disappointing, since, as shown in Table 1, the animals inoculated with tubercle bacilli which had been exposed to this salt of methylene blue all developed local tubercles in 18 days, much earlier than was the case with any of the other methylene blues, and death occurred in from two and one-half to five months in all cases, with marked general tuberculosis. The guinea-pigs used for treatment with this salt of

[1] *Jour. Infect. Dis.*, 1912, 11, p. 349.

the dye had been inoculated subcutaneously with a large dose of virulent human tubercle bacilli. Treatment was begun three weeks after inoculation, 0.1 per cent solution being employed and from 0.5 to 1.0 or more cubic centimeters being used at each dose.

1. Forty-one injections were given, making 33 mg. of the dye or 10.21 mg. of iodin. Death 149 days after inoculation. Large caseous gland in groin. Spleen and liver enlarged and showing necrotic areas. Lungs exhibit a few small tubercles. Some turbid fluid in abdomen, and pleural cavity filled with thick, semi-solid, yellowish-white exudate.

2. Twenty-four injections given in 53 days, totaling about 13 mg. of the dye or 4.02 mg. of iodin. Death 77 days after infection. Local glands slightly enlarged; not caseous. Spleen and liver enlarged and filled with tubercles and necrotic areas. Lungs not involved.

3. Twenty-five injections in 55 days, totaling 15 mg. of dye or 4.6 mg. of iodin. Died 79 days after inoculation. Two enlarged caseous glands in groin. Spleen and liver greatly enlarged and filled with tubercles and small necrotic areas. Lungs dotted with miliary tubercles.

4. Thirty-five dye injections made, totaling 20 mg. of dye or 6.18 mg. of iodin. Death 108 days after inoculation. Glands in groin enlarged and caseous. Liver enlarged, white, and hard, with a few necrotic areas. Spleen almost entirely necrotic. Lungs full of tubercles. Pleural cavity filled with serous exudate.

5. Forty-four injections given, totaling about 24 mg. of dye or 6.8 mg. of iodin. Animal died 140 days after infection. Local glands only slightly enlarged, not caseous. Spleen and liver very necrotic. Lungs dotted with small, hard, white nodules.

All tubercles turned blue on exposure to air or molybdate, with methylene blue iodid as well as with the chlorid.

After several experiments, earlier described, showing that cake pills of methylene blue could be fed to tuberculous guinea-pigs and that the dye administered in this way penetrated the tubercle, it was decided to try treating some tuberculous pigs with methylene blue by feeding the pills, since the subcutaneous injections, no matter how pure the preparation, nor how carefully graded the dose, nor how aseptically the injection is made, always in time and after many treatments cause serious ulcerations, which greatly increase the danger of secondary infections.

Each of six guinea-pigs first received a subcutaneous injection of 0.5 c.c. of a 0.3 per cent solution of medicinally pure methylene blue and each day afterward was fed a pill containing approximately 4 mg. of the dye. The strength of the pills was gradually increased up to 8 mg. per pill, or dose. Eight days after the first dye injection, each pig received a subcutaneous injection of 0.3 c.c.

of a dilute, filtered suspension of a three-weeks-old culture of human tubercle bacilli. Untreated controls died 75, 69, 80, 169, and 194 days after inoculation, all showing tuberculous lungs, generally necrotic spleens and livers, but none showing caseous glands at the site of inoculation.

1. Pig died 74 days after inoculation. Had received approximately 328 mg. of the dye by mouth. Local glands enlarged and caseous. Liver and spleen filled with tubercles and necrotic areas. Lungs packed with miliary tubercles and pleural cavities filled with serous exudate. Left adrenal showed a tubercle on anterior surface.

2. Death 80 days after inoculation after receiving about 360 mg. of dye by mouth. No local tubercle. Liver pale, yellowish in color, with a few deep-yellow areas. Spleen large, soft, deep red. No tubercles. Lungs nearly normal. Cause of death not determined, but not tuberculosis.

3. Death 101 days after infection. Spleen larger than normal and dotted with tubercles. Peribronchial glands large and lungs dotted with tubercles. Feeding was stopped some weeks before death.

4. Death 80 days after infection. Several enlarged and caseous glands in each axilla and in each groin. Liver and spleen enlarged and necrotic. Spleen completely necrotic—pale yellow—no normal spleen tissue. Lungs filled with tubercles.

5. Death 99 days after infection. Several enlarged and caseous glands in axilla. Spleen and liver enlarged and a mass of tubercles. Lungs only slightly involved.

6. Death 113 days after inoculation. Spleen one large necrotic mass. Liver full of necrotic areas. Lungs filled with miliary tubercles; peribronchial and retroperitoneal lymph glands enlarged.

In order to determine whether a single large injection of methylene blue given very soon after the infection of the animal would prevent the development of tuberculosis, four guinea-pigs were given, 24 hours after inoculation, 1 c.c. of a 0.5 per cent solution of methylene blue, two receiving it by subcutaneous injection at site of infection, and the other two intraperitoneally. One died on the eighth day, showing no sign of development of tuberculosis. The other three had developed local nodules by the twelfth day after infection and all developed a marked general tuberculosis.

Sellei[1] in 1912 published a report on the influence of dyes combined with poisons and therapeutic agents. Among other things, he states that, while the addition of methylene blue increases the toxic action of most copper salts, copper chlorid, which is the most toxic of these salts, becomes much less poisonous on the addition of methylene blue. One cubic centimeter of 1 per cent $CuCl_2$ to each 100 gm. body weight of animal injected subcutaneously kills guinea-

[1] *Biochem. Ztschr.*, 1913, 49, p. 466.

pigs in five to eight hours. Mixed with 0.3 c.c. of 1 per cent methylene blue, the animals live 12 to 16 days or longer. A similar diminution of toxic action of ferrous sulfate was obtained by mixing methylene blue with it, altho ferric chlorid with methylene blue kills much more quickly than the salt without the dye.

As the effect of numerous copper compounds on tuberculosis has been tested in this laboratory by Dr. Corper,[1] and as none of those tried has been found effectual, and as v. Linden and her associates have claimed good results from a combination of methylene blue with copper compounds, I inoculated eight guinea-pigs with human tubercle bacilli and 24 hours later gave four of them a subcutaneous injection of 2 c.c. of copper chlorid and methylene blue (25 c.c. of 1 per cent $CuCl_2$+3 c.c. of 1 per cent methylene blue). Twenty-four hours after infection the other four received a subcutaneous injection of 2 c.c. of ferrous sulphate+methylene blue (20 c.c. of 1 per cent $FeSO_4$+1 c.c. of 1 per cent methylene blue). Two of the pigs treated with $CuCl_2$ died within the first week showing no sign of tuberculosis. In all four there was very great infiltration and sloughing, so that no further injections were given and palpation would not reveal the presence of a tuberculous nodule. Two died early but the other two developed local and general tuberculosis. Of the four treated with $FeSO_4$ and methylene blue, one died on the eighth day with no sign of tuberculosis; another died 25 days after infection with two enlarged and partly caseous glands in groin. The other two exhibited local nodules on the twelfth day, which were discharging a caseous substance on the twenty-fifth day and died, one on the thirty-seventh and the other on the fifty-fifth day with marked generalized tuberculosis. Only one injection was given on account of the infiltration and ulceration following the first. These results do not suggest that a combination of these metallic salts with methylene blue can be used effectually or safely in the treatment of tuberculosis.

The results of treatment of experimental tuberculosis as shown by these tests are hardly as encouraging as one is led to expect from v. Linden's reports. She states that methylene blue penetrates the tubercle and stains the bacilli within the tubercle and even in

[1] *Jour. Am. Med. Assn.*, 1913, 60, p. 887.

the caseous centers of tuberculous lymph glands. This may easily be true, as it certainly penetrates the younger epithelioid tubercles, tho not often the centers of old, caseous tuberculous lymph glands. I, however, have never succeeded in finding blue-stained organisms in the tubercles of any of my animals, altho it would seem that, if they were as uniformly stained as is suggested by v. Linden's report, they might have been found in some of my treated animals. She also says that treatment with methylene blue chlorid or iodid causes healing of the initial sinus and abscess, increase of weight, lowering of fever, and prolongation of life, while 50 per cent of her six animals died from some cause other than the tuberculous infection, and one was entirely healed and sterile, as shown by animal inoculations. An absolute comparison of my results with hers is perhaps not fair, as she used a culture of low virulence, while mine was always a young, highly virulent culture. It is true that the guinea-pig is so susceptible to tuberculosis that therapeutic experiments on guinea-pig tuberculosis are more difficult and discouraging than on other animals or on man, but for that very reason we may infer that a therapeutic agent which heals the disease in the guinea-pig will be much more efficacious in man; but not if we facilitate the cure by using non-virulent cultures or those of low virulence. In my six feeding experiments, the initial sinus and abscess did discharge and dry up and heal either entirely or nearly. In one case a deep nodule later broke through the old, partly healed fistulous tract and it was discharging at the time of death. This might confirm one of v. Linden's contentions, were it not for the fact that in the five untreated controls of the same series the same thing occurred and in these no deep nodules formed, so that at death there were no local tubercles and the initial sinus was completely healed. Hence, this can hardly be ascribed to the methylene blue. In none of the other series was a healing of the local tubercle noted except in exceptional cases. The progressive weight and fever curve have not been systematically recorded in my cases, so that I cannot compare mine with hers in this respect. As to prolongation of life, Table 2 will epitomize my experience. Since it is fair to compare treated animals only with controls of the same series in which the same culture and doses were used, the results

have been tabulated in series. The first series had no definite controls and other factors entered in to modify results, as explained in the description of this series, but results will be tabulated so far as length of life is concerned.

TABLE 2.

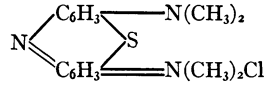
SERIES 1	SERIES 2				SERIES 3	
Medicinal Methylene Blue Days	Control Days	Medicinal Methylene Blue Days	Methylene Blue Iodid Days	New Methylene Blues Days	Control Days	Medicinal Methylene Blue Days
144	58	112	149	129 (R)	75	74
152	126	105	77	106 (GB)	69	80
133	138	112	79	133 (N)	80	101
186	231	89	108	100 (NSS)	169	80
131	.....	134	140	140 (GG)	194	99
130	.....	.....	.....	86 (NX)	.....	113
106	.....	.....	.....	99 (3R)	.....	.....
Average $140\frac{2}{7}$	$138\frac{1}{4}$	$110\frac{2}{5}$	$110\frac{3}{5}$	$113\frac{2}{7}$	$117\frac{2}{5}$	$91\frac{1}{5}$

A study of this table shows first that the duration of life, even in the controls of a single series, has so wide a range of variation that the conclusion is never justified that the process is less severe or is being favorably influenced by a given treatment simply because life is longer. Second, if we do attempt to compare averages, we find that the average duration of life of the treated animals is slightly less than that of the untreated of the same series, instead of being much greater, as v. Linden found it. As to her statement that three of her six animals died from some intercurrent affection instead of tuberculosis, altho they had that disease also, it is not at all unusual, even in guinea-pigs infected with tuberculosis, for death to be due to some cause other than the experimental disease in question. Guinea-pigs are susceptible to nearly all infections as well as to tuberculosis, and the tuberculous infection may even predispose them to some other fatal infection. For instance, 4 of the 7 pigs of my first series, 4 of the 17 of the second series, and 1 of the 6 in the third series, thus 9 of the 30 treated pigs, died, not from tuberculosis, but from some other condition, and in most of these the tuberculous involvement was very slight and not sufficient to be regarded as the cause of death. In many cases also, as in v. Linden's, such tubercles as were present, or at least some of the

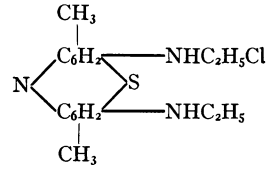
tubercles present, were hard and fibrous rather than caseous and might perhaps be regarded as in process of healing.

While there may have been some improvement in a few of the cases therefore, the results have not been so perfect or so uniform that methylene blue should be advised as a cure for tuberculosis, tho it may possibly have some palliative effect. The fact, however, that the dye seems to have some bactericidal power, either killing or markedly modifying the virulence of the human tubercle bacilli in nearly every case, makes it seem possible that this dye may serve as a starting-point for the development of a dye or chemical substance which may have a stronger and more specific influence on the tuberculous process than has methylene blue itself.

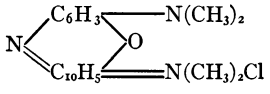
As reported, I have already used the iodid of methylene blue in place of the chlorid and have also used seven of the so-called new methylene blues which are but modifications of methylene blue. Methylene blue is a quinone imide thiazin dye, having the structural formula:



New methylene blues N and NSS are also quinone imide thiazin dyes, having the structural formula:



These dyes are no more efficient than methylene blue itself and are quite as irritating to the skin. New methylene blue GG is an oxazin instead of a thiazin dye and also replaces one C_6H_3 group by $C_{10}H_5$. The formula is as follows:



This dye penetrated the tubercle well, was fairly well borne, and the animal died of an acute peritonitis 140 days after inoculation, the tuberculous process being very slight. This dye and also

Capri blue, another oxazin derivative of methylene blue, will therefore be tested further. As to the other new methylene blues, I have not been able to find the formulae and most of them appear to be mixtures rather than pure dyes. They also are not promising as therapeutic agents.

In considering the question of feasible modifications of the methylene blue formula which could be made in the laboratory, the sulfur atom seemed naturally the first point of attack. Gosio[1] in 1905 suggested the use of sodium and potassium salts of selenium and tellurium as indicators of the life of bacterial cells, and Belfanti[2] in 1912 confirmed his results and extended his observations to the study of the tubercle bacilli. Both noted that, while 1 to 50,000 or 1 to 100,000 could be used with safety and would penetrate the bacteria and be reduced, a percentage higher than 1 in 10,000 was likely to kill the bacteria or at least inhibit their growth. Belfanti states that the tubercle bacilli, when actively living, become saturated with these salts and reduce them, and suggests that these salts of selenium and tellurium have a pronounced bacteriotropic power for the tubercle bacillus and could be used as the point of origin for new medicamentous preparations in the sense of Ehrlich. It was with this suggestion in mind that I decided to attempt to modify the methylene blue molecule by replacing the sulfur atom by an atom of selenium and also by one of tellurium, hoping in this way to combine the penetrating and reducing and bactericidal power of the selenium and tellurium with the milder bactericidal power and relative innocuousness of the methylene blue. The chemical processes were carried on for me by Dr. Walter Fraenkel; in the following I am giving his figures, and in the main his description of the method used by him. The same apparatus was used for making both dyes and is represented in Figs. 1 and 2.

A, Fig. 1, is filled with zinc and dilute hydrochloric acid. The hydrogen gas generated is dried in *B* with concentrated sulfuric acid. In *C* we place phosphorus selenide, made by fusing 11 parts of red phosphorus with 66 parts of finely pulverized selenium. Two grams of dimethylparaphenylendiaminechlorhydrate are dissolved in 200 c.c. of boiled, still hot water to which has been added 80 c.c. of concentrated hydrochloric acid. This solution is placed in flasks *D* and *E*, and *F* and *G* are filled half full of dilute permanganate solution, in order to avoid the escape of that portion of the

[1] *Ztschr. f. Hyg.*, 1905, 51, p. 65.

[2] *Ztschr. f. Chemother.*, 1912, 1, p. 113.

hydrogen selenide which has not entered into the reaction and the breathing of which is very dangerous. After a current of hydrogen gas has been passed through the apparatus to remove all the air, about 150 c.c. of sodium hydroxid is pressed with the help of a rubber bulb through the dropping funnel into the Erlenmeyer flask *C* and then allowed to stand some hours, with frequent shaking and slow passage of hydrogen gas, until the sodium hydroxid is colored dark brown. After the hydrogen stream has been cut down to a few bubbles to the minute, concentrated sulfuric acid is added drop by drop to the sodium hydroxid. The hydrogen selenide thus formed is allowed to pass through the apparatus for about 15 minutes. Then 60 c.c. of a 10 per cent solution of iron chlorid is added to *E* through the dropping funnel. After this has been well shaken, the rubber connections between *D* and *E* and between *E* and *F* are removed and *D* is connected with *F*. The fluid in *E* has now become green and is tested in a reagent glass to see whether it becomes darker green on the addition of more iron chlorid. If it does, iron chlorid is added gradually until tests do not become

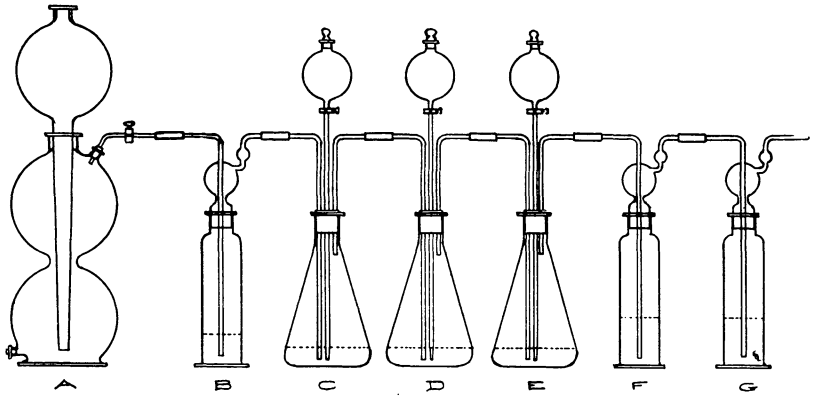
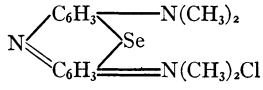


FIG. 1.—*A*, Kipp's apparatus; *B*, *F*, and *G* are wash bottles; *C*, *D*, and *E* are 500 c.c. Erlenmeyer flasks.

greener. The same is done with Erlenmeyer flask *D*. After it has been filtered with the aid of a suction pump, the fluid is placed in a shaking funnel and shaken with 100 c.c. of melted phenol. If, after standing, the two layers do not clearly separate from each other, a little concentrated hydrochloric acid is added. After the phenol is removed, the operation is repeated with new phenol and the two solutions mixed. This phenol solution is shaken four times with water, while each time after shaking a larger amount of hydrochloric acid is added to prevent the dye being taken up by the water. To the phenol solution are added 100 c.c. of ether and 100 c.c. of dilute hydrochloric acid and the mixture is strongly shaken. The dye then goes over into the water and the phenol-ether solution remains almost colorless. The dye solution is now shaken out several times with ether and finally allowed to stand over ether for 24 hours. After the separation of the ether and filtering, a part of the solution is placed in a small—not over 200 c.c.—distilling flask and distilled at a temperature of 30° to 40° to remove the water and hydrochloric acid. In order to have the boiling uniform and to prevent the entrance of air, carbon dioxid is conducted into the flask through a capillary tube, as shown in Fig. 2. After distillation, the lower part of the flask, containing the dye, is cut off, dried, and weighed; the dye is washed out with water and the flask dried and weighed. The difference gives us the amount of dye in the solution, the volume of which we measure.

This new dye is a blue dye, very soluble in water. It is not as stable as methylene blue; indeed it cannot be evaporated to complete dryness without breaking down. It is therefore necessary to keep the dye in water solution and even then the dye gradually breaks down, as shown by a precipitate in the bottom of the bottle. The formula for the dye is:



in which 21.6 per cent is selenium. The chemical reactions in the process of making this dye are the same as in the original method of making methylene blue used by Caro in 1876, hydrogen selenide being used instead of hydrogen sulfide.

In making the tellurium blue, flask *C* is carefully dried and in it is placed aluminium telluride, made by fusing four parts of pulverized tellurium with eight parts of aluminium. After hydrogen gas has been allowed to pass through the apparatus for some time, 30 per cent phosphoric acid is added drop by drop through the dropping funnel to the aluminium telluride. In this case, the oxidation is effected by means of hydrogen peroxid instead of by iron chlorid. After the addition of the hydrogen peroxid, the fluid is filtered as rapidly as possible and 100 c.c. of phenol added. The fluid and phenol are placed in a shaking funnel and shaken as often as the solution becomes blue. By this means, the blue dye is taken up by the phenol and the action of the hydrogen peroxid is stopped. This is repeated until the solution begins to become reddish, instead of blue. Then the phenol is poured off and purified in the manner described for the selenium blue. To remove the dye from the phenol, carbon tetrachlorid is used instead of the ether. It is then shaken with concentrated hydrochloric acid. (Very frequent shaking is necessary in order to get all the dye out of the phenol.) As the dye gradually becomes colorless, the completeness of the removal of the dye is tested by adding hydrogen peroxid to a small amount of the hydrochloric acid solution. The shaking is repeated until dark-blue color no longer follows the addition of the hydrogen peroxid. The rest of the treatment is the same as that for the selenium blue. This new dye is dark blue, soluble in water, even less stable than the selenium blue, cannot be completely dried, and even in water solution soon breaks down. Its formula is:

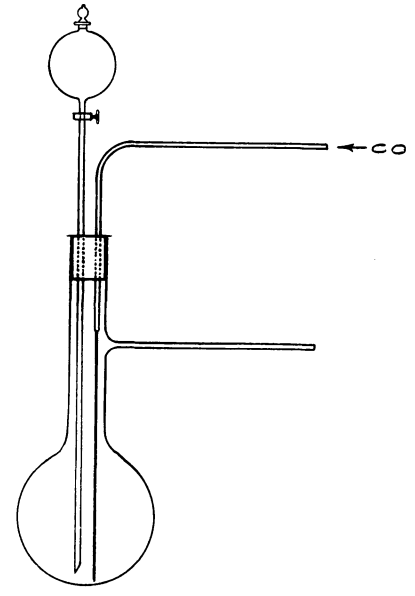
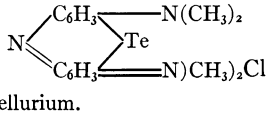


FIG. 2.



It contains 33.5 per cent of tellurium.

Both the selenium blue and the tellurium blue were, as stated before, rather unstable, especially the tellurium, and it was therefore necessary to use them as soon as possible after they were made. For this reason, the number of experiments is not so large as it might otherwise have been, altho of course more dye would have been made if the tests had been more encouraging. Both dyes were water-soluble, staining living tubercle bacilli fairly well, and fixed bacilli not so well. The stain, however, was never so clear and sharp as that obtained with pure methylene blue.

BACTERICIDAL ACTION OF SELENIUM BLUE AND TELLURIUM BLUE.

When these dyes were added to glycerin agar and human tubercle bacilli planted on the mixture, there was a more or less deep staining of the clumps which always failed to grow; in this respect, the new dyes resembled methylene blue. In the form of a very dilute, filtered suspension, human tubercle bacillus culture was also exposed for 24 hours to the action of 1 per cent solutions of these dyes, and one drop, diluted with 5 c.c. of normal salt solution, was injected subcutaneously into guinea-pigs, with the following results:

SELENIUM BLUE.

1. Large nodule found in axilla 18 days after inoculation. This soon ruptured, discharged, and nearly healed. Death 65 days after inoculation. Slight enlargement of axillary glands. Lungs, liver, and spleen show moderate involvement.

2. Eighteen days after inoculation, a discharging abscess was found at site of entrance of needle. This nearly healed. On thirtieth day, a deep nodule was found in axilla. Death about four months after inoculation. Enlarged and caseous gland in axilla. Lungs, liver, and spleen contained tubercles.

3. As in the other cases, nodule developed early before the eighteenth day and ruptured, discharged, and then healed. No glands became enlarged or caseous. Death occurred on the sixtieth day, lungs, liver, and spleen being full of miliary tubercles.

4. Again an initial abscess formed, discharged, and healed. On sixtieth day a deep nodule was found in axilla. Death on ninety-fifth day. Enlarged and caseous gland in axilla. Peribronchial and retroperitoneal glands enlarged. Liver, spleen, and lungs full of tubercles and necrotic areas.

TELLURIUM BLUE.

1. Abscess developed, discharged, and healed within the first month. On sixtieth day a small nodule was found in axilla. Pig was killed 158 days after inoculation, because it was weak and emaciated and hind parts were paralyzed. No enlarged or

caseous glands in axilla. Lungs showed a few small, translucent areas and one cavity-containing tubercle. Liver normal. Spleen showed a few small tubercles. Extent of tuberculous process hardly sufficient to account for condition.

2. Abscess ruptured and discharged early. On sixtieth day a deep nodule was discovered in axilla. Died on one hundred and thirty-third day. Lungs, liver, and spleen contained tubercles. Caseous gland in axilla.

3. Initial abscess ruptured and healed early. Death on one hundred and eighty-ninth day. No local tubercle. Lungs, liver, and spleen contained tubercles. Thorax full of serous exudate.

4. Initial abscess ruptured and healed early. Deep nodule found on sixtieth day. Death 125 days after inoculation. Enlarged and caseous gland in axilla. Peribronchial and retroperitoneal glands enlarged. Lungs, liver, and spleen contained tubercles.

A comparison of these with the controls of the same series shows no or very little bactericidal power in these dyes, as may be seen from Table 3. The average duration of life is in favor of the tellurium blue.

TABLE 3.

Dyes Used	Initial Abscess	Local Tubercle Days	Death	Postmortem Changes
Control....	18 days. Discharged and healed	31	140 days	Local and general tuberculosis
	18 days	32	4 months	" " " "
	18 days. Discharged and healed	61	75 days	" " " "
	18 days. Healed	31	4 months	" " " "
Selenium blue.....	18 " "	Very late	65 days	Slight local and general tuberculosis
	18 " "	31	4 months	Local and general tuberculosis
	18 " "	None	60 days	No local; marked miliary tuberculosis of organs
	18 " "	60	95 "	Local and general tuberculosis
Tellurium blue.....	18 " "	60	158 " (killed)	No local and slight general tuberculosis
	18 " "	60	133 days	Local and general tuberculosis
	18 " "	60	189 "	" " " "
	18 " "	60	125 "	" " " "

AVERAGE DURATION OF LIFE AFTER INOCULATION.

Controls	Selenium Blue	Tellurium Blue
112 days	100 days	151 days

THERAPEUTIC EXPERIMENTS WITH THE SELENIUM AND TELLURIUM BLUES.

Trial experiments with guinea-pigs having advanced tuberculosis showed that these dyes had about the same effect on the skin as did methylene blue and also that they penetrated the tubercle, were reduced there, and could be oxidized to a blue dye by action of 10

per cent ammonium molybdate. In other words, they behaved very much like methylene blue, but were stronger toxins and weaker dyes. On account of the effect on the skin and the very small dose that could be given subcutaneously, the method of feeding with cake pills was used in the series treated with these new dyes. As in the series fed with methylene blue the animals first received one subcutaneous injection followed by daily feedings. Eight days after the first injection each animal received a subcutaneous injection of 0.3 c.c. of a dilute, filtered suspension of human tubercle bacilli and the daily feeding of the pills was continued.

1. An abscess was found ruptured and discharging 19 days after inoculation. This healed and a deeper gland in axilla gradually enlarged. Died 68 days after inoculation. No autopsy.

2. An abscess developed by the nineteenth day after inoculation. This later ruptured, discharged, and healed. No other local tubercle developed. Death 94 days after infection. Liver normal. Spleen large and necrotic; lungs a mass of miliary tubercles.

3. A discharging abscess found on nineteenth day. This partly, but never entirely, healed. Death 81 days after infection. Slightly enlarged and slightly caseous gland in axilla. Liver pale and filled with necrotic areas. Spleen enlarged and filled with miliary tubercles. Apices of both lungs showed many advanced tubercles, while the rest of lungs had only a few small tubercles.

4. Nodule at point of inoculation on nineteenth day. This discharged and healed. Death 95 days after infection. No local tubercles. Liver and spleen large, soft, and deep red. No tubercles visible. Lower lobes of both lungs solid and filled with miliary tubercles.

5. Nineteen days after inoculation, a discharging nodule was found at point of inoculation. This crusted over and partly healed, but broke open again to emit discharge from deeper tuberculous glands. Death on ninety-sixth day. Both axillae contained numerous large, caseous, tuberculous glands. Liver, spleen, and lungs filled with tubercles of different ages. Especially in lungs, many of the tubercles were caseous with hard periphery. Pericardium filled with serous exudate.

6. Nineteen days after infection, a discharging nodule was found at point of inoculation. This failed to heal and later glands in both axillae and in one groin were found enlarged. Death on the eighty-seventh day. Caseous lymph glands in axillae and in groin. Spleen and liver large, hard, and full of tubercles and necrotic areas. Lungs filled with miliary tubercles. Pleural cavities filled with serous exudate.

7. Nodule found at site of inoculation on nineteenth day. Did not discharge and heal. Death 68 days after inoculation. Enlarged and caseous gland in axilla. Lungs, liver, and spleen filled with miliary tubercles. Very marked generalized tuberculosis.

8. Nineteen days after inoculation, a discharging nodule was found at site of inoculation. This later nearly healed, but several deeper glands in axillae enlarged

and became caseous. Death about four months after infection. Lungs, liver, and spleen full of tubercles.

The pills used in the above series contained from 2 to 3 mg. of selenium blue and a single pill constituted a daily dose. The next series was fed with pills containing each from 1.5 to 2 mg. of tellurium blue.

1. No local tubercle developed, but the pig became emaciated and died on the thirty-seventh day after infection. No enlarged glands in axilla, but lungs were filled with miliary tubercles; spleen and liver exhibited a few tubercles and a few small necrotic areas.

2. Eighteen days after infection, a discharging nodule was found at point of inoculation. This healed and deeper nodules were found 32 days after inoculation. Death on sixty-seventh day. Glands in both axillae slightly enlarged, with beginning caseation. Liver pale, lobulated, and showing several large areas of necrosis. Spleen large and necrotic with numerous tubercles of different sizes. Lungs filled with large, hard tubercles. Pericardium filled with cloudy, yellowish fluid.

3. Eighteen days after infection, a large hard nodule was found at site of injection. No healing. Other deeper nodules developed. Death 91 days after infection. Large ulcer in right axilla and under it the tissue was dense and granular. Spleen and liver large and filled with tubercles. Lungs packed with large opaque caseous tubercles. Pericardium filled with cloudy fluid.

4. Discharging nodule found in axilla on eighteenth day. This partly healed. Death about four months after inoculation. Spleen, liver, and lungs filled with tubercles.

5. Discharging nodule at site of injection found on eighteenth day. No healing. Death 94 days after inoculation. Raw ulcer on right side of chest wall. Enlarged and caseous glands in both axillae and in both groins. Lungs filled with miliary tubercles. Liver enlarged containing many yellowish-green patches of necrosis; spleen somewhat enlarged and necrotic along lower border.

6. Large, tense nodule found at point of inoculation on eighteenth day; this soon ruptured and partially healed. Deep nodules developed late. Death 90 days after infection. Ulcer and several caseous nodules in axilla. Spleen and liver enlarged and filled with tubercles and necrotic areas. Lungs filled with tubercles, many being caseous and others clear and hard.

7. A discharging nodule was found at site of injection on eighteenth day. This healed and deeper nodules developed. Death about four months after inoculation. Local glands enlarged and caseous. Liver, spleen, and lungs filled with tubercles.

8. Large tense nodule found at site of injection on eighteenth day. This discharged and healed. Death about four months after infection. Liver, spleen, and lungs filled with tubercles.

A comparison of the duration of life after infection of the treated as compared with the untreated animals and of the methylene blue as compared with the new dyes is summarized in Table 4.

All of the animals represented in this table received the same amount of the same culture of human tubercle bacilli and all the treated animals were fed with cake pills of the different dyes as before described.

TABLE 4.

Controls Days	Methylene Blue Days	Selenium Blue Days	Tellurium Blue Days
75	74	68	37
69	80	94	68
80	101	81	91
169	80	95	120
194	99	96	94
.....	113	87	90
.....	.....	68	120
.....	.....	120	120
Average 117.4	91⅛	88⅝	92½

CONCLUSIONS.

1. Methylene blue will penetrate the tubercle, stain the living tubercle bacillus, and in some cases kill the bacillus *in vitro* and in others lessen its virulence. When added to the culture media, a relatively small percentage of methylene blue will inhibit the growth of the human tubercle bacillus.

2. Methylene blue iodid is no less irritant than the chlorid and has less bactericidal power and no greater therapeutic value.

3. The new methylene blues are various modifications of the methylene blue molecule and have in the main no advantage over methylene blue. New methylene blue GG, however, showed some effect in the one case in which it was used therapeutically and it, with other oxygen derivatives of methylene blue, will be given further tests.

4. Selenium blue and tellurium blue are new blue dyes made in this laboratory, in which the sulfur of the methylene blue molecule is replaced by selenium and by tellurium. They are weaker and less stable dyes than methylene blue and more toxic and less bactericidal than that dye. They penetrate the tubercle and are reduced in it and can be reoxidized; they stain the living tubercle bacillus, but more faintly than does methylene blue. In fact, they behave in all respects as weaker editions of methylene blue and have no advantage over it.

5. Neither methylene blue nor any of the allied dyes tested by me may be said to have much therapeutic influence over experimental tuberculosis of the guinea-pig. While methylene blue seems for many reasons a favorable starting-point for tuberculosis chemotherapy, other modifications of it and probably many others must be tried before we can claim to have found a specific for this disease.

In closing, it gives me great pleasure to express my gratitude to Dr. Fraenkel for his intelligent and skilful co-operation with me in making the new dyes used in this work, and to Miss Sherman for her tireless assistance in all the experimental routine work.